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MICROBIOLOGICAL ANALYSES OF TEST RIG HEAT EXCHANGER TUBES

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Heat exchanger tubes removed from the Pickering and Darlington heat exchanger test rigs were analyzed for the presence of viable microorganisms. A diverse microbial community became established on the shell side of the tubes within days following exposure of the tubes to lakewater. The numerically dominant microbial groups included aerobic heterotrophic bacteria, pseudomonads and actinomycetes. In the Pickering test rig, their densities approached steady-state population levels one to two weeks following tube installation. Sulphate-reducing bacteria, widely implicated in the corrosion of several types of heat exchanger materials, were present in relatively low abundance within the surface deposits on the tubes.

INTRODUCTION

The Chemistry and Metallurgy Department of Central Nuclear Services is evaluating the performance of various heat exchanger materials using an experimental test rig at Pickering NGS. The test rig simulates the process conditions that exist within a moderator heat exchanger and will aid in the identification of factors influencing material performance (Doherty 1988). The potential importance of microbiologically-induced corrosion within the heat exchanger environment prompted the quantification of specific physiological groups of microorganisms in the biofilm adherent to the tubes. In addition, four heat exchanger tubes from a similar test rig that is in operation at Darlington NGS were recently removed for microbiological analyses at the request of the Corrosion and Tritium Technology Section (Metallurgical Research Dept) (Dunstall 1988). Funding for the Darlington analyses was provided by the CANDU Owners Group (COG). The tubes that have been analyzed from both test rigs represented various exposure periods, the longest of which slightly exceeded one year.

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Physiological groups of microorganisms are broadly classified according to their ability to grow on highly specific media which simulate certain environmental conditions. The groups enumerated included aerobic heterotrophic bacteria, pseudomonads, actinomycetes, ammonifying bacteria, sulphate-reducing bacteria (SRB) and fungi. Preliminary results of these analysis are presented for tubes removed from untreated (i.e. no biocides) test rig bundles. Additional tubes are to be removed from the Pickering test rig in 1988 and results for this entire study will be presented in a final report.

METHODS AND MATERIALS

Heat exchanger tubes removed from the test rigs were immersed in lakewater from the rig (Pickering) or in sterile phosphate buffer (Darlington) within alcohol-sterilized PVC containers. These containers were designed such that contact with the fouled tube surface was avoided. Tubes were promptly shipped to Research Division where they were maintained at 4°C overnight. All tubes removed from the Pickering rig have been NO8800 except for one Monel-400 tube and one NO8825 tube.

Microbiological analysis were performed the morning following collection using aseptic technique during all sample processing stages. Excess buffer was allowed to drain from the tubes following their removal from the containers. A 1-cm wide swath of adherent material was scraped from the tube. The total surface area of the scrapes varied from 18 to 64 cm^2. This material was washed from the tube using phosphate buffer (18 mL), into a pre-weighed sample bottle. The weight of the material scraped from the tube was determined. Two mL of this initial suspension was added to 18 mL of phosphate buffer giving a ten-fold dilution. Subsequent transfers (2 mL in 18 mL buffer) provided a dilution series with a concentration range down to 10^{-7} of the initial suspension.

Aliquots from the initial solution and each of the ten-fold serial dilutions were transferred to selective media. Pour plates of specific agar medium were inoculated with 0.1 mL of sample which was dispersed evenly over the agar surface using an alcohol-flamed glass spreader. Each dilution was plated in triplicate for each medium type, and plates were inverted and incubated. Nutrient agar supplemented with 1% glucose was used for the cultivation of aerobic heterotrophs, a broad class of organisms with rather diverse nutritional requirements. Pseudomonads, which are a specific type of aerobic heterotroph, were cultured on Flo-agar medium. Plates for both groups were incubated at 25°C for 48 h prior to enumeration.

Fungi were isolated on Czapex Dox agar, poised at pH 5.5 that was supplemented with tellurite solution (0.036% w/v) to inhibit bacterial growth. Plates were incubated at 21°C for 10 days. Actinomycetes were cultured on starch-casein medium supplemented with cycloheximide to inhibit fungal growth and were incubated at 30°C for 10 days.

Anaerobic sulphate-reducing bacteria were isolated into freshly prepared Postgate's medium (Postgate 1979). These bacteria require an environment with a redox potential of -100 mV or lower which was achieved by incorporating thioglycollate into the medium. Test tubes were inoculated with 1 mL of sample. Five deep tube cultures, prepared from each dilution, were incubated at 30°C and evaluated for growth for a period of up to 21 days. Blackened medium indicated the conversion of ferrous sulphate to ferrous sulphide by sulphate-reducing bacteria.

Organic nitrogen compounds are dissimilated by a wide variety of heterotrophic microorganisms, yielding ammonia and other end products. Test tubes containing 4% peptone solution were inoculated with 1 mL of sample and incubated for 48 h at 30°C. Five test tubes were inoculated from each dilution. The presence of ammonifying bacteria was indicated by a positive reaction for ammonia; ie. the development of a bright yellow colouration following the addition of a drop of culture medium to a drop of Nessler's reagent on a glass slide.

Enumeration Procedures

Counts of between 30 and 300 colonies per petri plate were used to quantify densities of specific groups of microorganisms using the assumption that each colony developed from one cell (American. Public Health Association 1975). Numbers within this range strike a balance between high variability associated with low colony counts and over-crowding observed when colonies are abundant. Conversion of count data to density per unit surface area was obtained from:

$$\text{Count} = \frac{\text{Initial Diluent Volume (18 mL)}}{\text{Inoculum Volume (0.1 or 1.0 mL) [OR Transfer Volume (2 mL)]}} \times 10^{(\#\ \text{serial dilutions})} \times \frac{1}{\text{Inoculum Vol (0.1 or 1.0 mL)}} \times \frac{1}{\text{Surface Area of Scrape (cm}^2\text{)}}$$

The most-probable number method was used to estimate densities of ammonifying and sulphate-reducing bacteria (Alexander 1982). Complete descriptions of culture and enumeration procedures are given in Sharpe (1983b, 1984).

RESULTS

Counts for each group of microorganism, for tubes periodically removed from the Pickering test rig over a period of up to 375 days, are shown in Table 1. The actinomycetes have, in general, been the most numerous microbial group with slightly lower abundances of aerobic heterotrophs and pseudomonads. These three groups have comprised more than 99.8% of total microbial numbers on individual heat exchanger tubes. The sulphate-reducing bacteria and ammonifyers were the least abundant of the six microbial groups enumerated. Numerical abundance of the fungi is also low although their cell size is very much greater than that of bacteria.

Among the aerobic heterotrophic bacteria, pseudomonads and actinomycetes there was a rapid increase in the numbers of attached microorganisms in the Pickering rig during the first week or two following tube placement as shown in scatter plots in Figure 1. After this period the rate at which bacterial numbers increased was much lower as microbial populations appeared to approach asymptotic levels indicative of steady-state conditions. This consistency in the abundances of these three microbial groups existed even though there is a general increase in deposit thickness with duration of exposure in the test rigs. The relatively low abundance, particularly for aerobic heterotrophs and actinomycetes, observed for the Pickering tube removed after a one year exposure, was of interest since this has been the only Monel-400 tube examined. The distinctive feature of Monel-400 is the presence of

TABLE 1

Densities of Microorganisms on Heat Exchanger Tubes
Removed from the Pickering Test Rig, (Cells/cm^2)

Alloy	Treatment	Date	Exposure [Days]	Wt. Deposit [mg/cm2]	No. cells per cm2					
					Aerobic Heterotr	Pseudomonads	Ammonif	Fungi	Actinom	Sulphate-Reducers
Water	L. Ont. *	Sep 2/87	0	N/A	1.65E+06	1.72E+06	1.10E+05	1.00E+03	2.03E+06	2.20E+01
NO8800	Bckg	Sep 9/87	0	neg	8.00E+00	0.00E+00	0.00E+00	7.50E+01	0.00E+00	0.00E+00
NO8800	None	Sep 2/87	4	neg	1.01E+04	1.01E+04	6.32E+03	4.50E+01	2.74E+04	4.00E+00
NO8800	None	Sep 9/87	11	6.4	4.97E+04	5.63E+04	2.46E+03	4.50E+01	4.72E+04	1.20E+01
NO8800	None	Sep 23/87	25	7.4	1.06E+04	8.74E+03	1.76E+02	0.00E+00	6.23E+03	4.00E+00
NO8800	None*	Oct 22/87	60	neg	5.01E+03	3.51E+03	2.40E+01	9.00E+00	1.68E+04	4.00E+00
NO8800	None	Feb 10/88	166	20.3	5.41E+04	6.64E+04	4.50E+01	6.50E+01	3.92E+05	6.00E+00
NO8800	ocl-*	Feb 17/88	173	neg	3.35E+02	2.04E+02	1.00E+00	0.00E+00	1.32E+03	1.00E-02
NO8800	None	Feb 11/88	6	2.1	1.77E+03	1.53E+03	2.00E-01	0.00E+00	3.09E+03	1.00E-02
NO8800	None	Feb 17/88	11	3.2	5.11E+03	1.04E+03	3.70E+00	0.00E+00	1.17E+04	7.00E-02
NO8800	ocl-*	Mar 9/88	33	0.6	3.51E+02	1.12E+02	2.00E-02	0.00E+00	1.02E+03	4.70E-01
NO8800	None	Mar 9/88	33	20.9	6.78E+03	4.77E+03	6.00E+00	0.00E+00	1.99E+05	4.60E+00
NO8800	None	Apr 11/88	66	19.3	1.09E+04	7.57E+03	7.00E-01	0.00E+00	5.67E+03	2.40E+00
NO8800	None	Apr 11/88	66	neg	2.01E+03	5.67E+03	1.60E+00	4.80E+00	1.10E+05	1.80E+00
sludge	No cm-3 *	Apr 11/88	66	N/A	4.51E+05	8.85E+05	2.14E+04	2.44E+02	1.47E+06	1.46E+03
Mon400	None	May 25/88	~365	neg	3.42E+04	3.05E+04	4.90E+00	0.00E+00	6.99E+03	4.90E+00
NO8800	None	July 4/88	311	neg	2.59E+05	1.84E+04	7.50E+00	3.30E+01	8.72E+04	2.90E+00

*Not considered in this presentation.

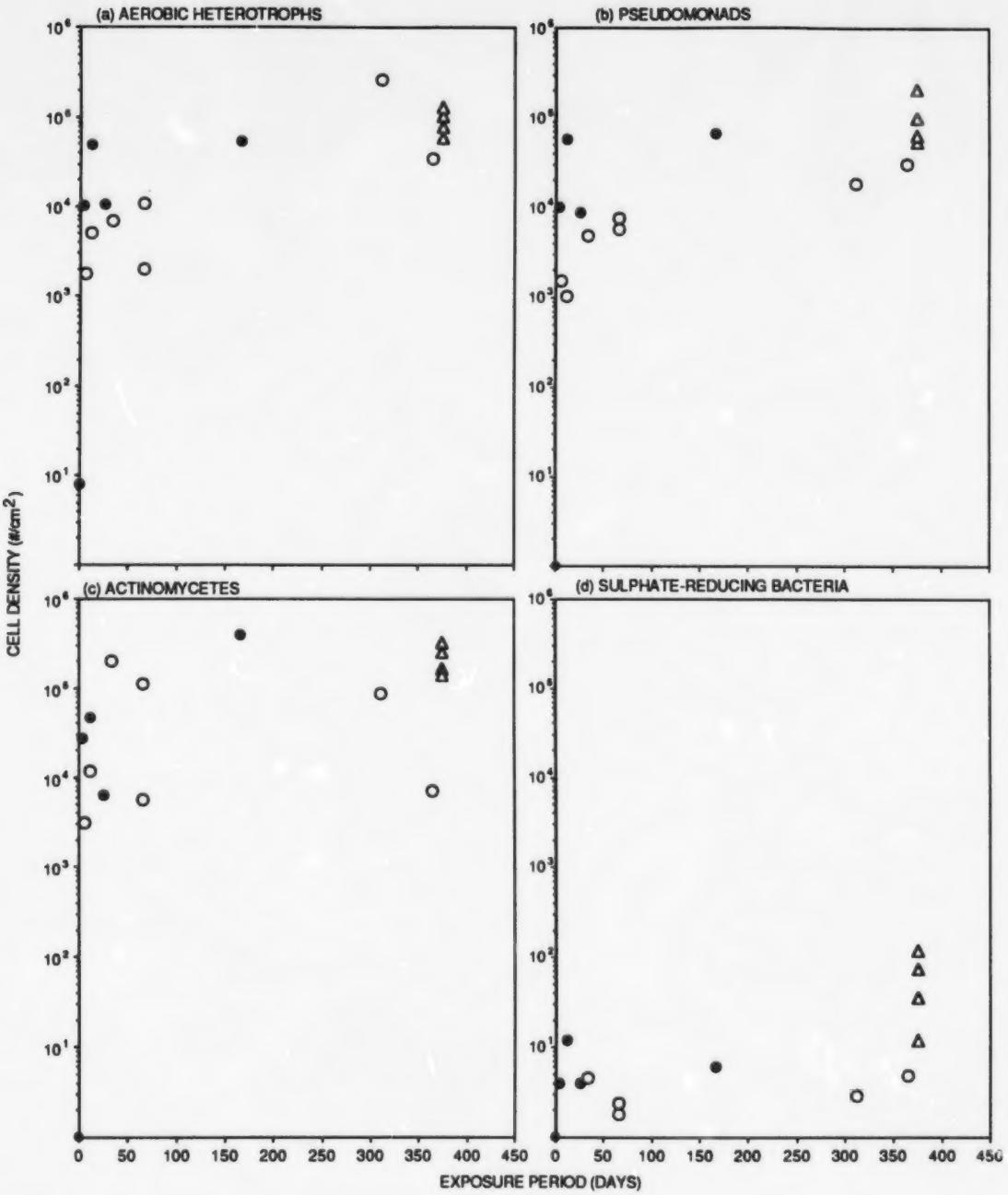

FIGURE 1
ABUNDANCE (#/cm^2) OF (a) AEROBIC HETEROTROPHS, (b) PSEUDOMONADS, (c) ACTINOMYCETES AND (d) SULPHATE-REDUCING BACTERIA ON TEST RIG HEAT EXCHANGER TUBES FOLLOWING EXPOSURE PERIODS OF UP TO 375 DAYS

LEGEND: △ - DARLINGTON TEST RIG
● - PICKERING TEST RIG (1987)
○ - PICKERING TEST RIG (1988)

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copper (33%) which is fairly readily leached from the metal oxide (A. Brennenstuhl, per. comm.).

Results were converted to cell number per gram deposit (for tubes with a measurable deposit weight) to enable further comparison of the test rig data to previously conducted laboratory studies (Sharpe 1984, 1986). This compilation of data suggests that there is a rapid increase in the abundance of each of the three major microbial groups following initial exposure of metal to lakewater (Figure 2). This period of colonization is followed by a slower rate of increase in cell number. Data for the four Darlington tubes indicates the existence of steady-state population levels. Among these tubes, which had all received the same exposure, abundances of the different microbial groups varied by less than a factor of four, with the exception of the SRB's (factor = 10). In the long-term laboratory study (420 days) tubes immersed in Lake Ontario water had relatively low numbers of aerobic heterotrophs compared to abundance on test rig tubes.

Sulphate-reducing bacteria were present in low abundance (generally $\leq$ 12 cells/cm^2) on the Pickering tubes but were somewhat more abundant on the Darlington tubes (12 to 120 cells/cm^2) (Figure 1d). These results suggest that the generally longer exposure period and a more established surface deposit contributed to higher SRB abundance among the Darlington tubes.

Comparison of test rig and laboratory data for the SRB's, with results expressed on a per gram deposit basis, indicated that the abundance of SRB's increased rapidly following exposure to lake water (Figure 2d). Subsequently a slower rate of increase in cell numbers occurred and, for the Darlington tubes, SRB numbers appeared to have attained a steady state level. In the long-term laboratory study, heat exchanger tubes with a natural biofilm layer alone or one which was covered with a pure silica deposit had SRB densities that were generally comparable to those of the Darlington tubes; ie. about 10^4 cells/g deposit. In contrast, overlaying the biofilm layer with a sediment deposit (ashed to remove the organic content) resulted in the development of SRB numbers to approximately 10^6 cells/g deposit. These results were comparable under both oxygenated and stagnant test conditions (Sharpe 1986).

Although the abundances of ammonifiers (50 to 120/cm^2) and fungi (130 to 440/cm^2) were similar for the four Darlington tubes, no overall trend with time was observed when these results were compared to Pickering data. Variable results were obtained for ammonifiers on the Pickering tubes while fungi numbers were generally low (0 to 75/cm^2) and they were often absent.

DISCUSSION

The microbial analyses of test rig heat exchanger tubes has helped to characterize one aspect of the environment under which pitting corrosion may occur. Microbes that had colonized the surface of the HX tubes were sampled since the relationship between densities of bacteria in lakewater and those within the biofilm is tenuous (Sharpe 1983c, 1984, Costerton et al. 1988). Densities of organisms on surfaces tend to be much higher than in lakewater.

King (1987) found that initial film formation on the Darlington test rig heat exchanger tubes had occurred within 3 weeks and was similar in appearance for 4 different alloys. The initial deposit was believed to be calcium carbonate and other minerals. Results from the Pickering test rig have shown that microbes colonize metal surfaces within days following their exposure to lakewater. The microbial community within the biofilm deposit on the heat exchanger tubes is influenced by environmental factors such as temperature, pH, silt

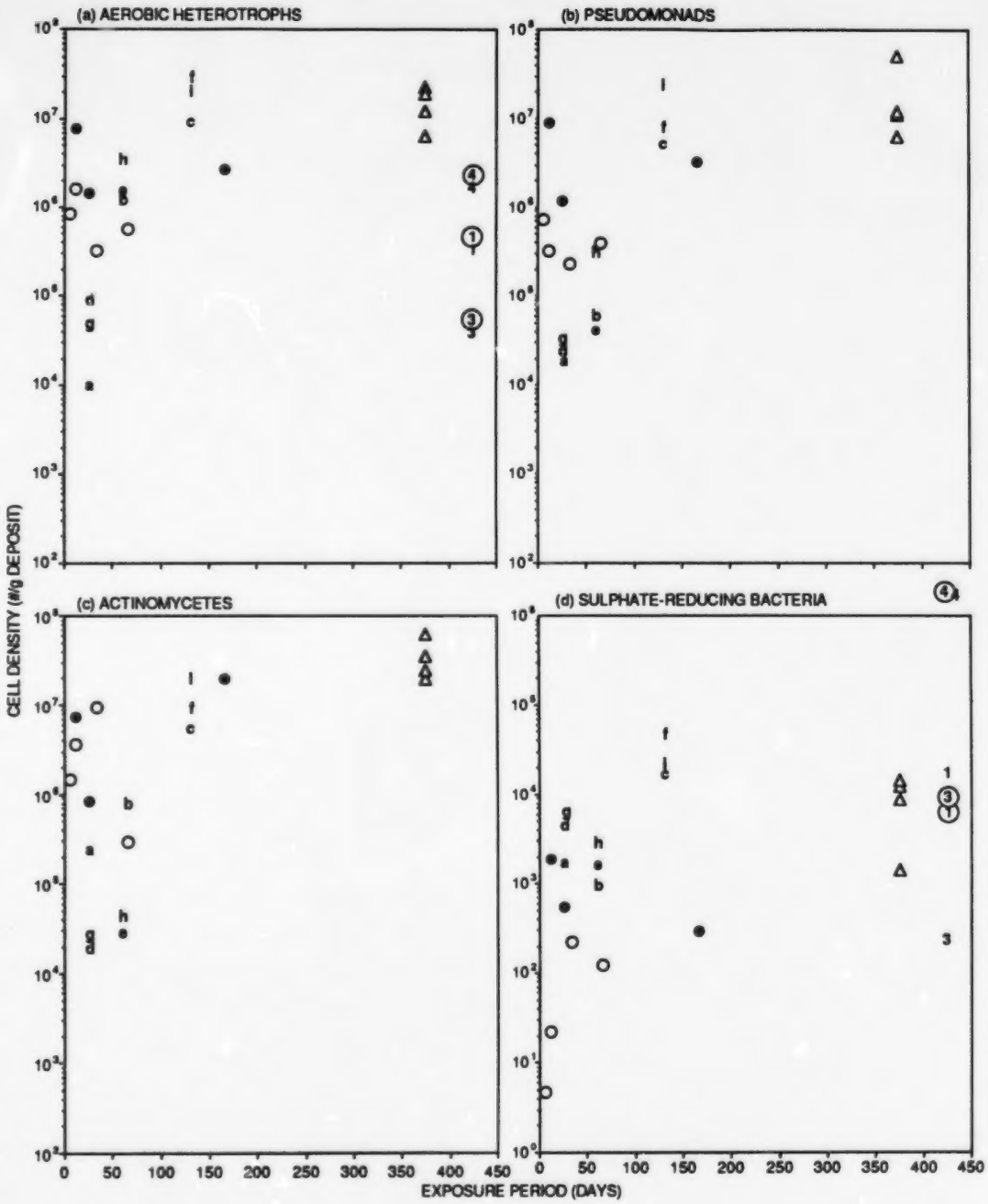

FIGURE 2

ABUNDANCE (#/g DEPOSIT) OF (a) AEROBIC HETEROTROPHS, (b) PSEUDOMONADS, (c) ACTINOMYCETES AND (d) SULPHATE-REDUCING BACTERIA WITHIN BIOFOULED SURFACES FROM TEST RIG AND LABORATORY IMMERSION STUDIES

LEGEND: TEST RIG STUDIES - △ DARLINGTON, ● PICKERING (1987) AND ○ PICKERING (1988)
IMMERSSION STUDIES - a TO l (OHRDR 84-276-K). a - c \ LAKE HURON (BRUCE) WATER;
d - f LAKE ERIE (NANTICOKE) WATER; g - l LAKE ONTARIO (PICKERING) WATER ON 304L SS
- 1,3 & (OHRDR C886-3-K) 1 BIOFILM + SILICA; 3 BIOFILM ONLY 4 BIOFILM + LAKE DEPOSIT
UNDER OXYGENATED OR STAGNANT (CIRCLED #'S) CONDITIONS FOR NO8800

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load and chemical and biological composition of the lakewater, all of which may vary seasonally. There was some indication that the aerobic heterotrophs and pseudomonads became more rapidly established on the Pickering tubes when installed in August (i.e. 1987) as opposed to February (i.e. 1988) (Figure 1).

The predominance of actinomycetes, pseudomonads and aerobic heterotrophs in the biofilm attached to test rig heat exchanger tubes was also observed on stainless steel wafers in laboratory studies with lakewater used as the immersion medium (Sharpe 1984). Comparisons were possible for the aerobic heterotrophs. Following the colonization stage, densities of this group on the test rig tubes was approximately 10^5 cells/cm^2 while densities during immersion tests were higher, ranging from 3.6 x 10^5 to 1.2 x 10^6 cells/cm^2. Epifluorescence microscopy on NO8800 heat exchanger tubes exposed to Lake Ontario water in the laboratory indicated that total (live and dead) cell density had reached 2.0 x 10^7 cells/cm^2 after 50 days exposure (Sharpe 1985). In a detailed analyses of biofilm composition following immersion testing in polluted river water, Tarbuck and Wyborn (1980) found aerobic heterotrophs dominated the microbial community with densities of up to 10^{10} cells/cm^2 reported.

Once established on the heat exchanger tubes, the number of aerobic heterotrophs on a deposit weight basis was about 10^7 cells/g deposit. This was comparable to the 1.5 x 10^6 to 2.0 x 10^7 cells/g deposit in the Pickering NGS "B" moderator heat exchanger (Sharpe 1983c) but much higher than densities (2.1 x 10^3 to 3.3 x 10^4 cells/g deposit) in a process feedwater line at the Bruce Heavy Water Plant (Sharpe 1983a). Both of these site locations had been subject to corrosion.

Sulphate-reducing bacteria were identified on the basis of their ability to reduce ferrous sulphate to ferrous sulphide. The presence of SRB's on the heat exchanger tubes indicates that the potential for their development exists in areas depleted of oxygen (ie. anaerobic), with a greater likelihood of corrosion occurring. Estimates of SRB densities do not necessarily provide a reliable measure of activity within the biofilm (Cragnolino and Tuovinen 1984).

Densities of active SRB required to initiate pitting corrosion is uncertain. Sharpe (1986) described extensive discolouration of heat exchanger tubes that had 1.6 x 10^6 SRB's/g deposit in the biofilm plus sediment deposit treatments of her 14-month immersion experiments. A pitted process feedwater line at BHWP was found to contain 4.9 x 10^3 to 1.6 x 10^5 SRB/g dry wt. deposit with greatest abundance at sites where corrosion had occurred (Sharpe 1983a). A failed moderator heat exchanger bundle (NO8800) at Pickering NGS "B" had between 2.4 x 10^4 and 3.7 x 10^5 SRB/g dry wt. deposit (Sharpe 1983c). Densities expressed on the basis of deposit wet weight would be somewhat lower than results expressed on a dry weight basis, but within the range of about 10^3 to 10^5 cells/g deposit. Nodules that had developed on the stainless steel surface of a water box contained 8.5 x 10^5 to 6.2 x 10^7 SRB/g deposit (Hayner et al. 1988). Costerton et al. (1988) described a vigorous biofilm population that contained fewer than 10 SRB's/cm^2 with densities of up to 10^6 cells/cm^2. The abundance of SRB's on the Darlington tubes (~10^3 to 10^4 cells/g deposit or 12 to 120 cells/cm^2) would therefore appear to be on the lower end of the range implicated in corrosion. Densities of SRB's for the Pickering tubes, which generally have been in service for a shorter period of time, were mostly lower than this indicated range.

Although the abundance of SRB's appeared to be related to the age of the deposit no such relationship existed with respect to the thickness of the deposit. This is not particularly surprising since considerable difficulty is encountered in attempting to quantify the amount of deposit. Much of the deposit build-up was lost when the tubes were removed from the test rig. Furthermore, samples were not taken randomly, or over the entire surface of the tubes, but rather were selected to include a portion of the heavier deposit layers. It should be noted, however, that the number of microorganisms per unit weight of deposit is a valid measure and only subject to large inaccuracies when a marginal amount of deposit has been removed from a tube. Attempts to scrape thin deposits from the tubes results in the absorption of pre-weighed wash water onto the unscraped portion of the deposit. This loss of wash water results in abnormally low values for the weight of deposit.

The abundances of ammonifiers or fungi per unit surface area were relatively consistent among the Darlington tubes. However, among the tubes removed from the Pickering test rig following various exposure periods, the abundances of these two groups fluctuated considerably with time. These results suggest that the ammonifyers and fungi are transient components of the biofilm, possibly reflecting recent conditions in the water environment such as variations in organic content.

Care should be exercised in interpreting results based on cell cultivation procedures. Selective media allow for the growth of bacteria that may, or may not, be physiologically active within the biofilm. Similarly, other groups that are active within the biofilm may be under-represented following cultivation. The mere presence of a microbial community does not necessarily imply active involvement in the corrosion process. Specific types of physiological activity or metabolic by-products (ie. sulphides, phosphides) contribute to corrosion as described by Pankhania (1988) for the sulphate-reducing bacteria. Sampling of heat exchanger tubes tends to involve the scraping of a relatively large tube surface area. However, bacterial distributions within biofilms tend to be patchy. The possible concentration of cells in pockets of relatively intense metabolic activity is not readily apparent when measured cell densities are low (Johnson and Longaker 1987). Patchy cell distributions within the biofilm can lead to differential aeration cells, galvanic couples and ion concentration cells by chelation of metals from the water, or from the direct utilization of metal through metabolic redox reactions (Hughes 1978). These factors may have serious implications for materials susceptible to pitting corrosion.

Confirmation of a cause and effect relationship requires that the association between microbes (presence and physiological activity) and the corrosion site be demonstrated. In laboratory studies, Sharpe (1986) found that inorganic material alone (ie. no bacteria) or the presence of a biofouling layer alone on the metal surface had little or no effect on the initiation of corrosion in NO8800 tubes after a 14 month exposure period. However, a close correlation was apparent between the extent of metal discolouration, indicative of the early stages of corrosion, and numbers of SRB's under both aerobic and stagnant conditions. Understanding the mechanisms by which bacteria facilitate corrosion will lead to opportunities for the development of control measures.

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